# Application of IR (infrared) microspectroscopy as a novel tool to biochemically signature the effects of chemical pollutants in mammalian cells


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*University of Lancaster*

PATET OMNIBUS VERITAS

# My talk: a story in three parts

- Introduction to infrared (IR) spectroscopy and computational analysis
- Example demonstrating how potentially powerful this approach is
- My findings and future applications

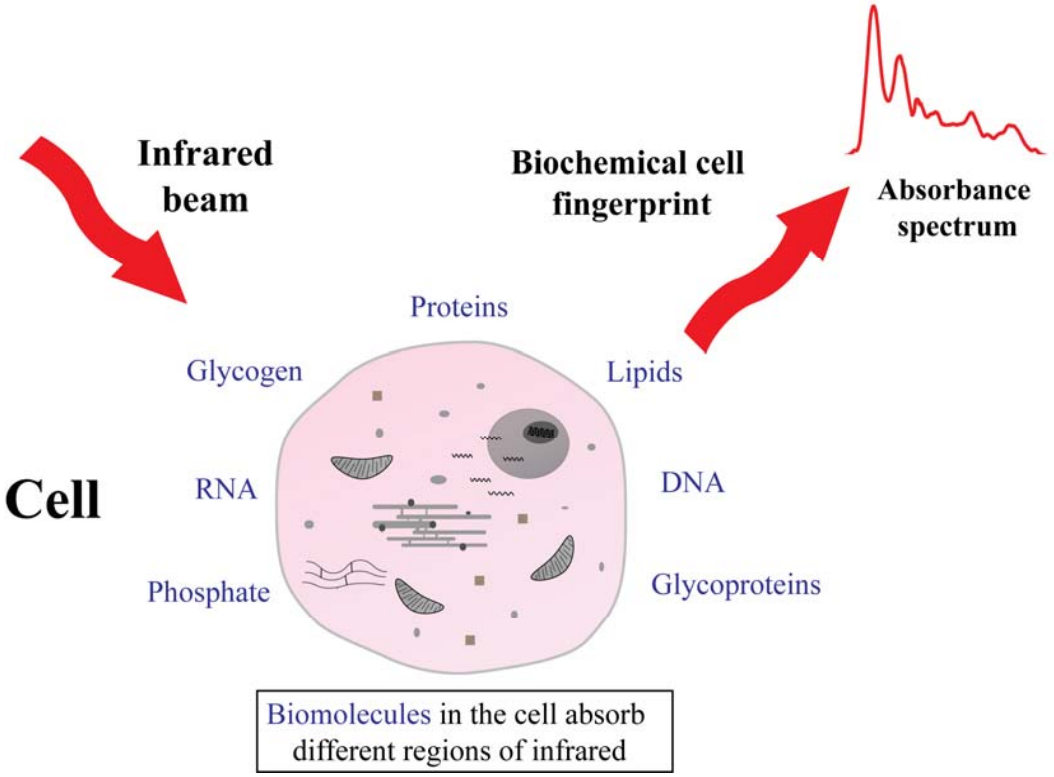
# Introduction: types of IR spectroscopy

- Fourier Transform (FTIR) - transmission
- Photothermal - heat
- Attenuated total reflection - attenuated beam

- vary only in methods of signal detection

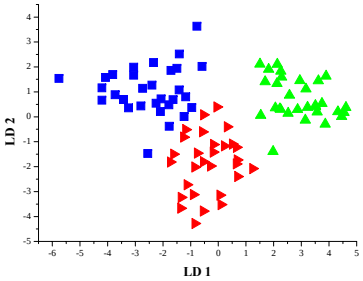
→ "Biochemical-cell fingerprint"

# An overview of our application

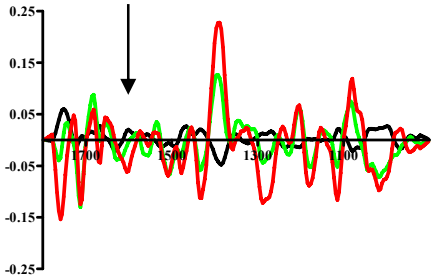



## Computational analysis

### Scores plot

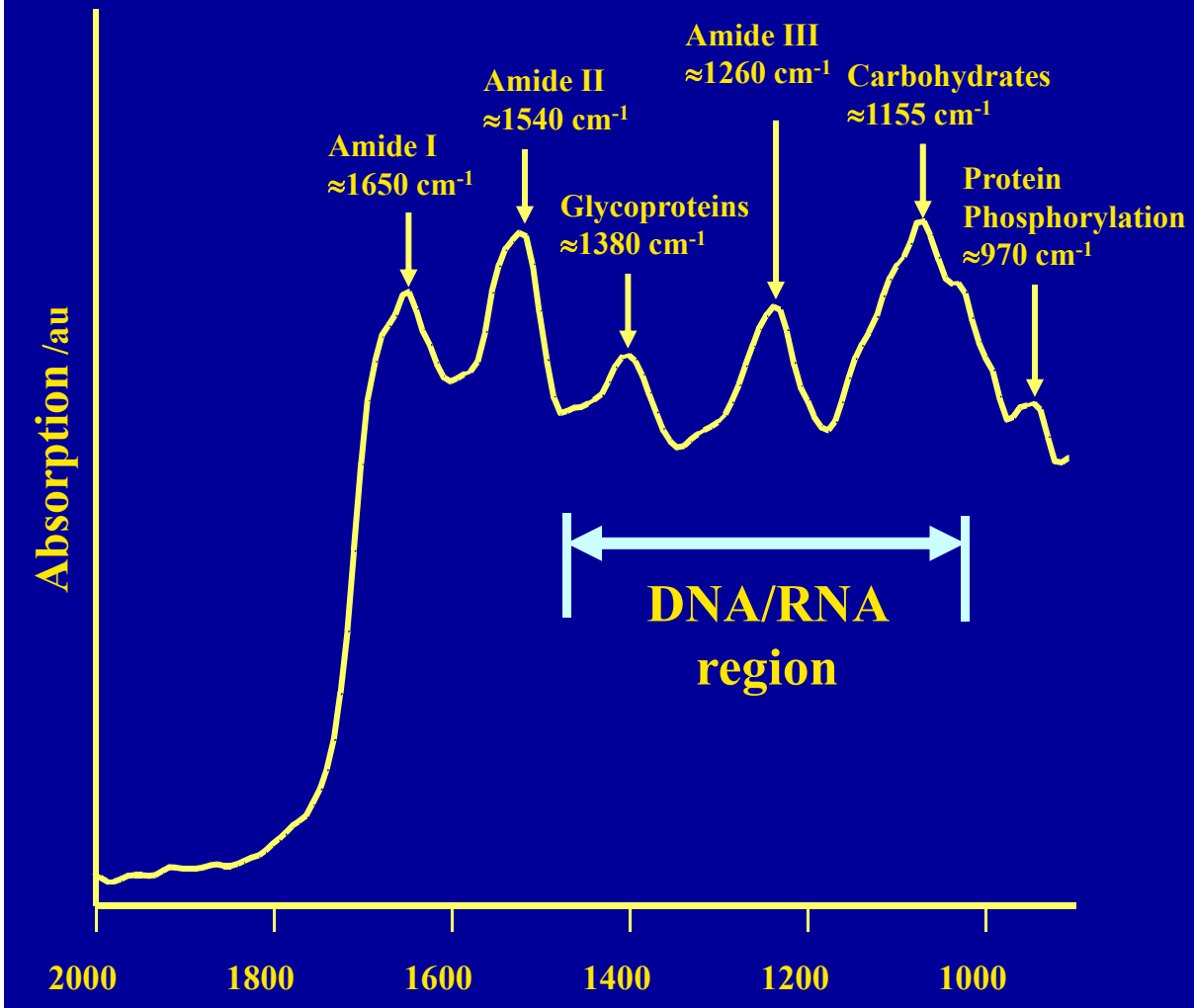



### Loadings plot




**Each of these cellular components ⇒ a specific IR biomarker**

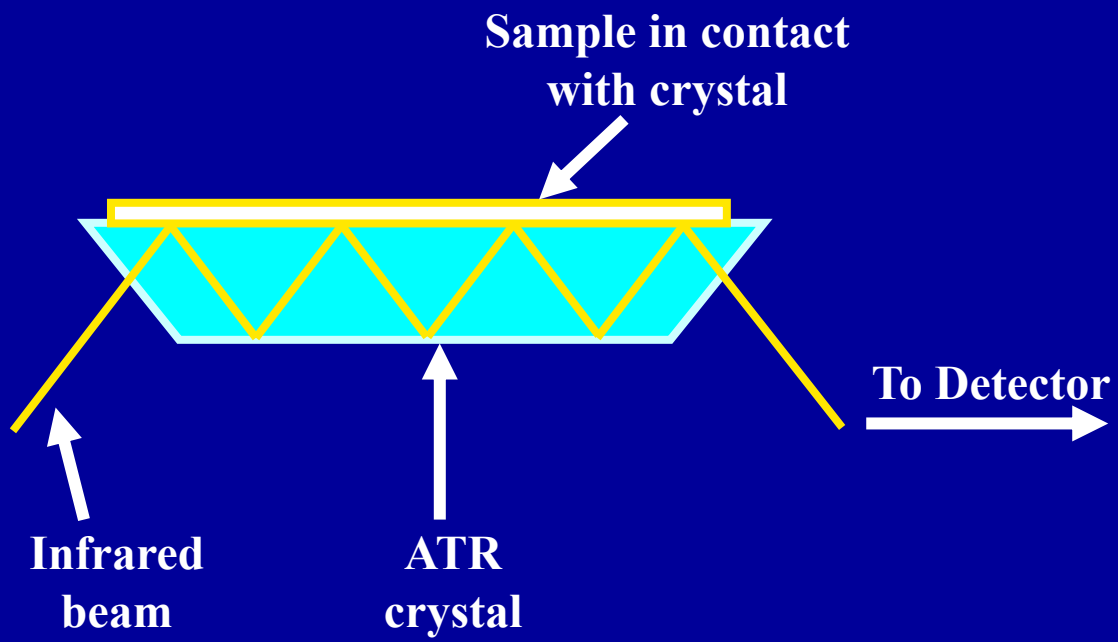
# Cell markers detectable by IR spectroscopy




*Biophysical Journal* (2005) 88: 3699-3706

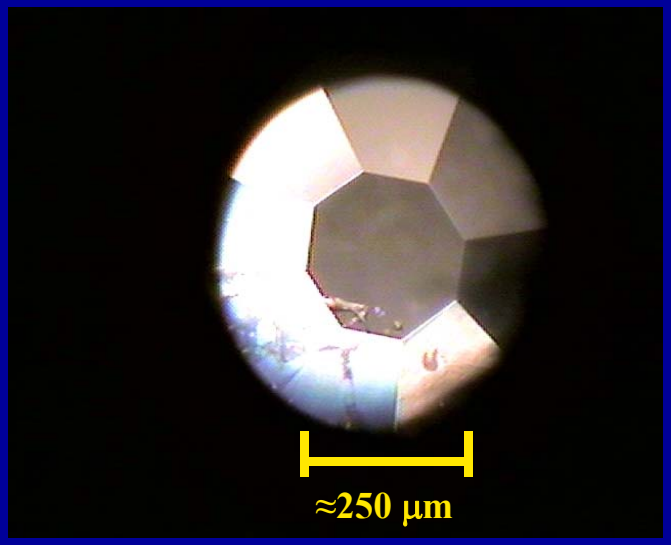
- $NH_2$ scissoring vibrations of the nucleotide bases (≈1680 $cm^{-1}$)
- $CH_2$ scissoring and $CH_3$ asymmetric bending vibrations of lipids, proteins and nucleic acids (≈1450-1480 $cm^{-1}$)
- Weak NH vibrations and CH in-plane deformations of nucleic acids (≈1450-1300 $cm^{-1}$)
- $PO_2^-$ asymmetric (≈1225 $cm^{-1}$) and symmetric (≈1084 $cm^{-1}$) stretching vibrations of nucleic acids and phospholipids
- Ribose-phosphate main-chain vibrations (≈1050 $cm^{-1}$)
- Integrated absorbance ≈900-1200 $cm^{-1}$ → ? a predictor of malignancy

# Attenuated Total Reflection (ATR) FTIR spectroscopy



# IR transparent crystal

**Crystal before application to cells**



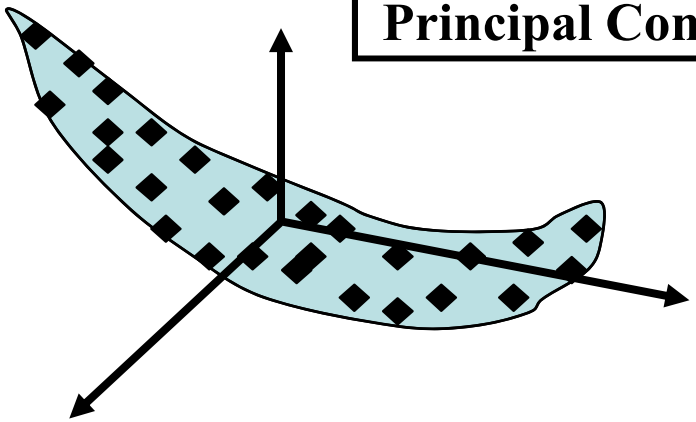

**Crystal after application to cells**

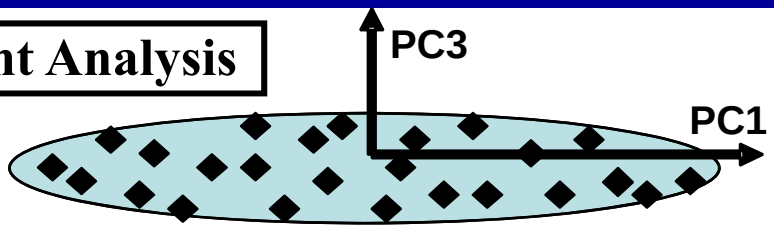
# Principal component analysis (PCA)

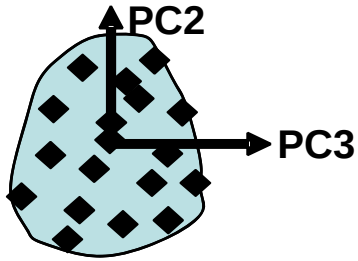
- Each spectrum is derived from *one* analysis
- Each spectrum contains *many* variables; PCA reduces this to *one* point (score) in a scores plot
- Scores are rotated along coordinates known as Principal Components (PCs); nearness implies similarity and separation signifies dissimilarity
- Typically, just *two or three* PCs are required
- The loadings plot for each PC highlights the wavenumbers responsible for variance in a particular rotation of *all* the samples
- PCA-LDA is used to reduce intra-class variation and maximize inter-class variance

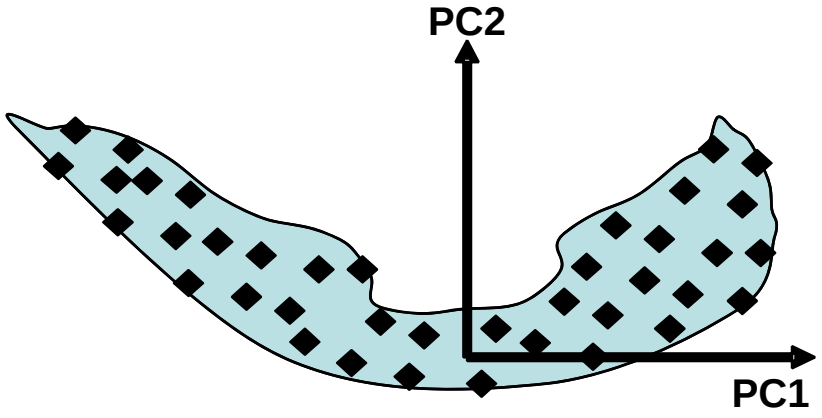
Allows one to visualise how samples CLUSTER according to DIFFERENCES in their IR spectra

# Principal Component Analysis






**Example = rotating a banana to see the bite**

# Example of the power of this approach

*Environ. Sci. Technol.* **2007**, *41*, 5915–5922

## Infrared Spectral Analysis of MCF-7 Cells Treated with Serum-Lipid Extracts Segregates Predominantly Brominated Flame Retardant-Exposed Subjects from Those with Mainly Organochlorine Exposures

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*Biomedical Sciences Unit, Department of Biological Sciences, Lancaster University, Lancaster LA1 4YQ, U.K., State Key Laboratory of Organic Geochemistry, Guangdong Key Laboratory of Environment and Resources, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, P.R. China, Department of Environmental Toxicology, School of Public Health, Guangxi Medical University, Nanning, Guangxi 530021, P.R. China, Institute of Environmental Pollution and Health, School of Environment and Chemical Engineering, Shanghai University, Shanghai 200072, P.R. China, and Centre for Chemical*

contaminant profiles in the extracts showed that polybrominated diphenyl ethers (PBDEs) accounted for 46.0% of total organohalogens and were higher than dichlorodiphenyl-trichloroethanes (DDTs) in Region E; DDTs were the major contaminants (85.2% of total contaminant load) compared to PBDEs (8.7%) in Region S. These results suggest that ATR microspectroscopy can segregate cell-biochemical effects as a consequence of very different exposure paradigms.

### Introduction

Persistent organic pollutants (POPs) tend to be resistant to degradation through photolysis, chemical, and/or biological transformation (*1*). Many organisms are subjected to long-term exposure to accumulating levels of POPs, the effects of which remain to be ascertained (*2*, *3*). Through the generation of an infrared (IR) spectrum, a "biochemical cell fingerprint" may be generated, and such applications have immense potential in toxicological assessment (*4*). Combined with data handling approaches, such as principal component analysis (PCA), that allow for the reduction of large spectroscopic datasets toward cluster analysis, there is now the possibility to derive a biomolecular signature of a cell or even a subcellular compartment. It is then possible to rapidly discriminate differing IR spectra and to identify the wavenumbers, and thus the molecular alterations, responsible (*5*).

Fourier-transform infrared (FTIR) microspectroscopy may be employed toward identifying cells in different states (*6*). Vibrational spectra consist of peaks that correspond to different molecular bonds (Figure 1); the main ones are amide

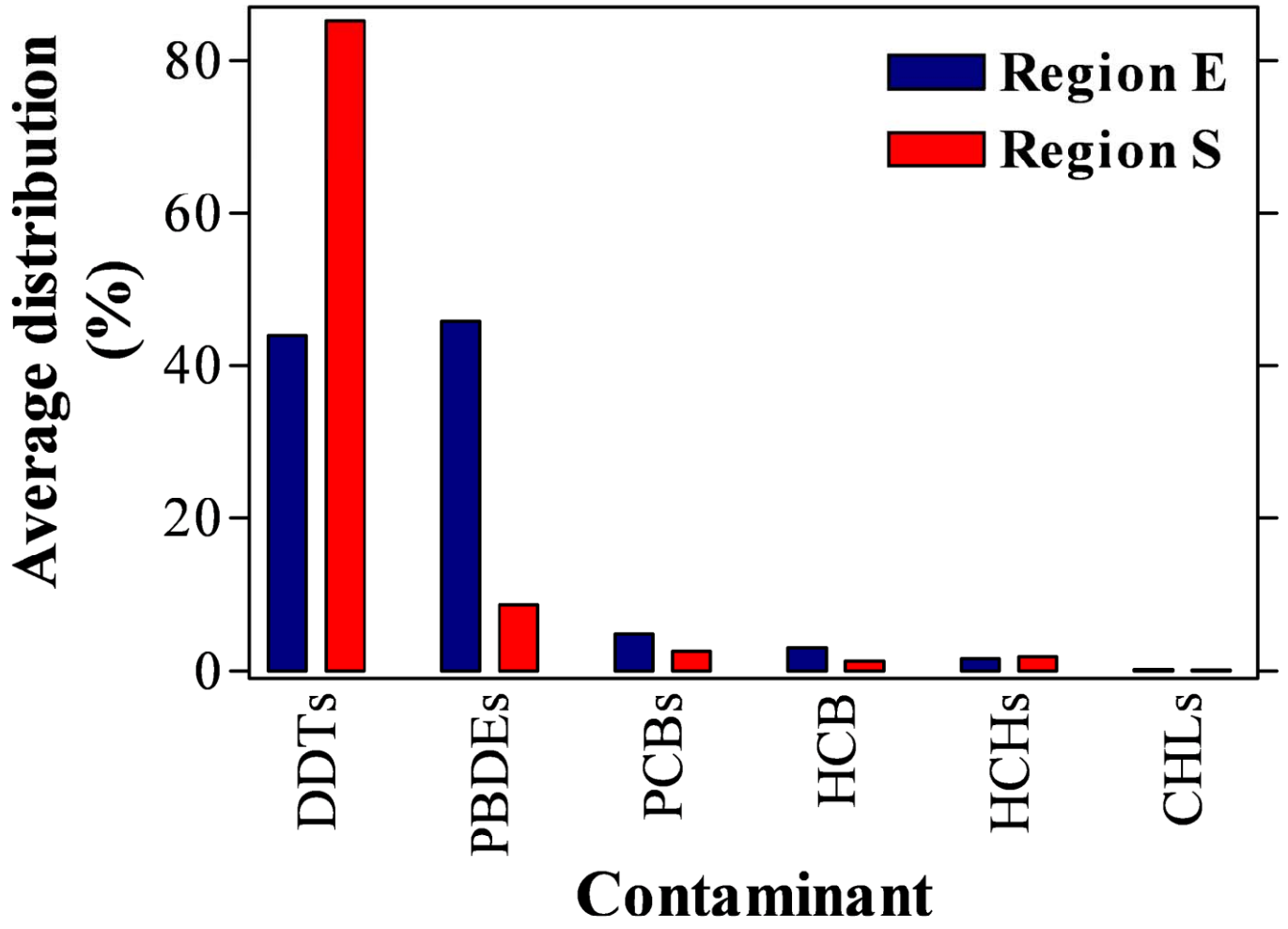
# ATR FTIR spectroscopy to signature different exposures

**Serum lipid samples were obtained from:**

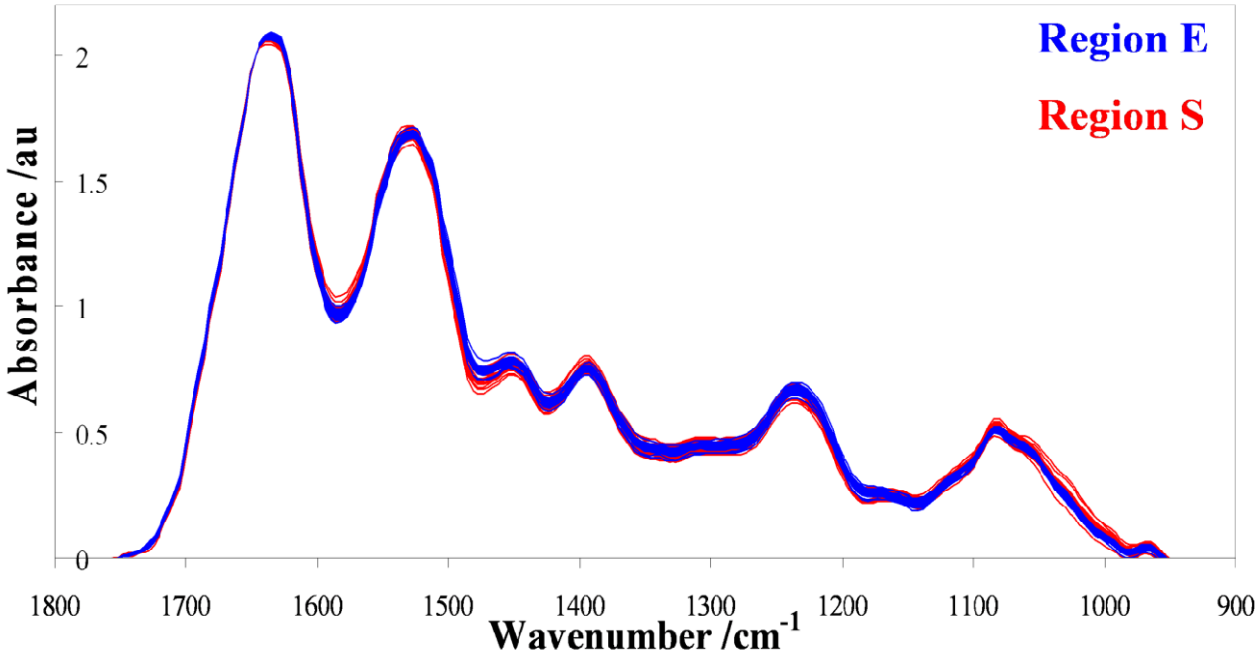
1. **Residents living in electronic waste (e-waste) region (Region E) in the south of PR China**
2. **Residents from nearby fishing village (Region S)**

- **MCF-7 cells were treated for 24-h with ≤5 mg-equivalent lipid extracts**
- **IR spectra of treated-cell populations were obtained**

# Contaminant profile

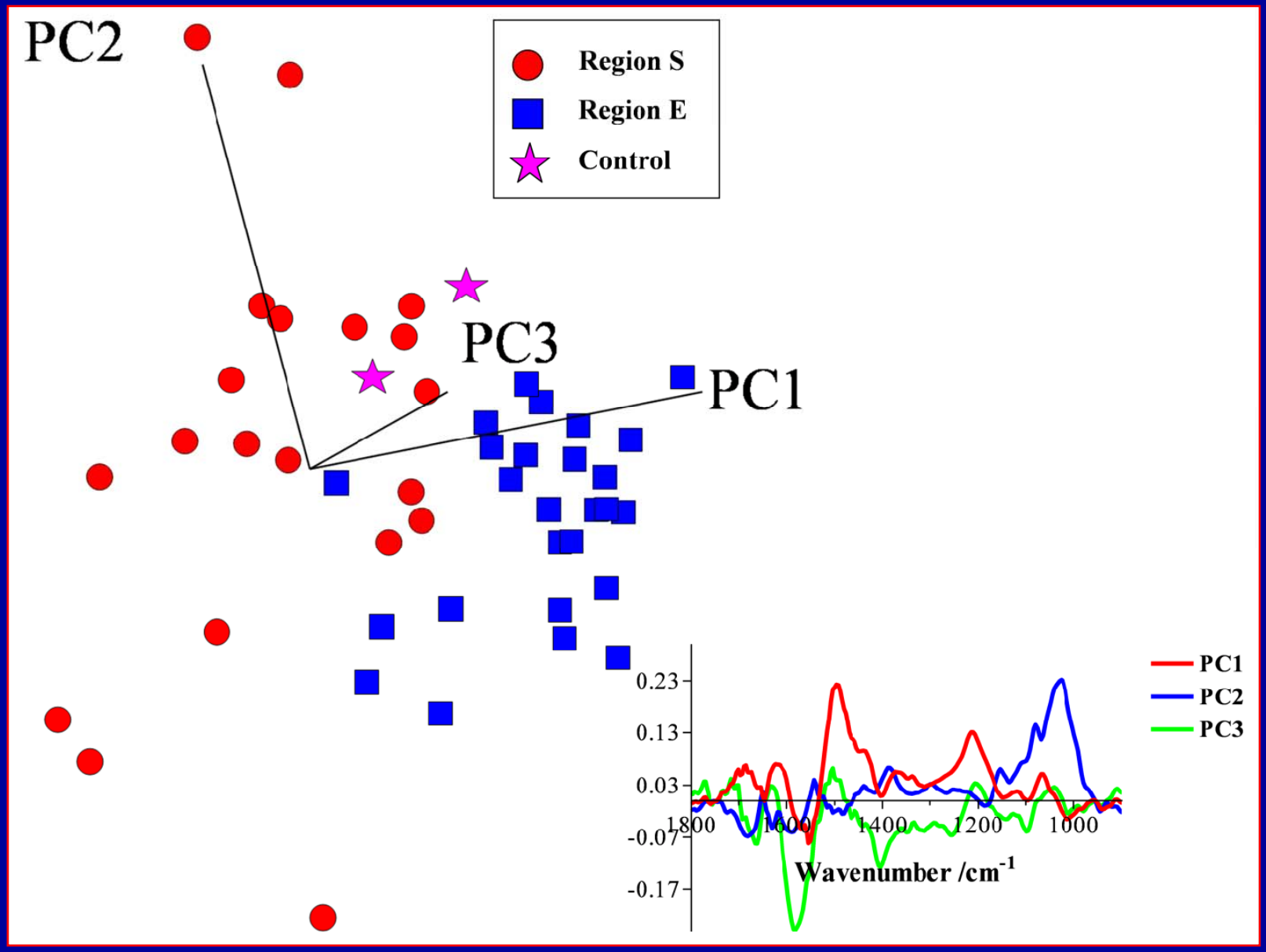


# Difficult to identify differences between IR spectral groups

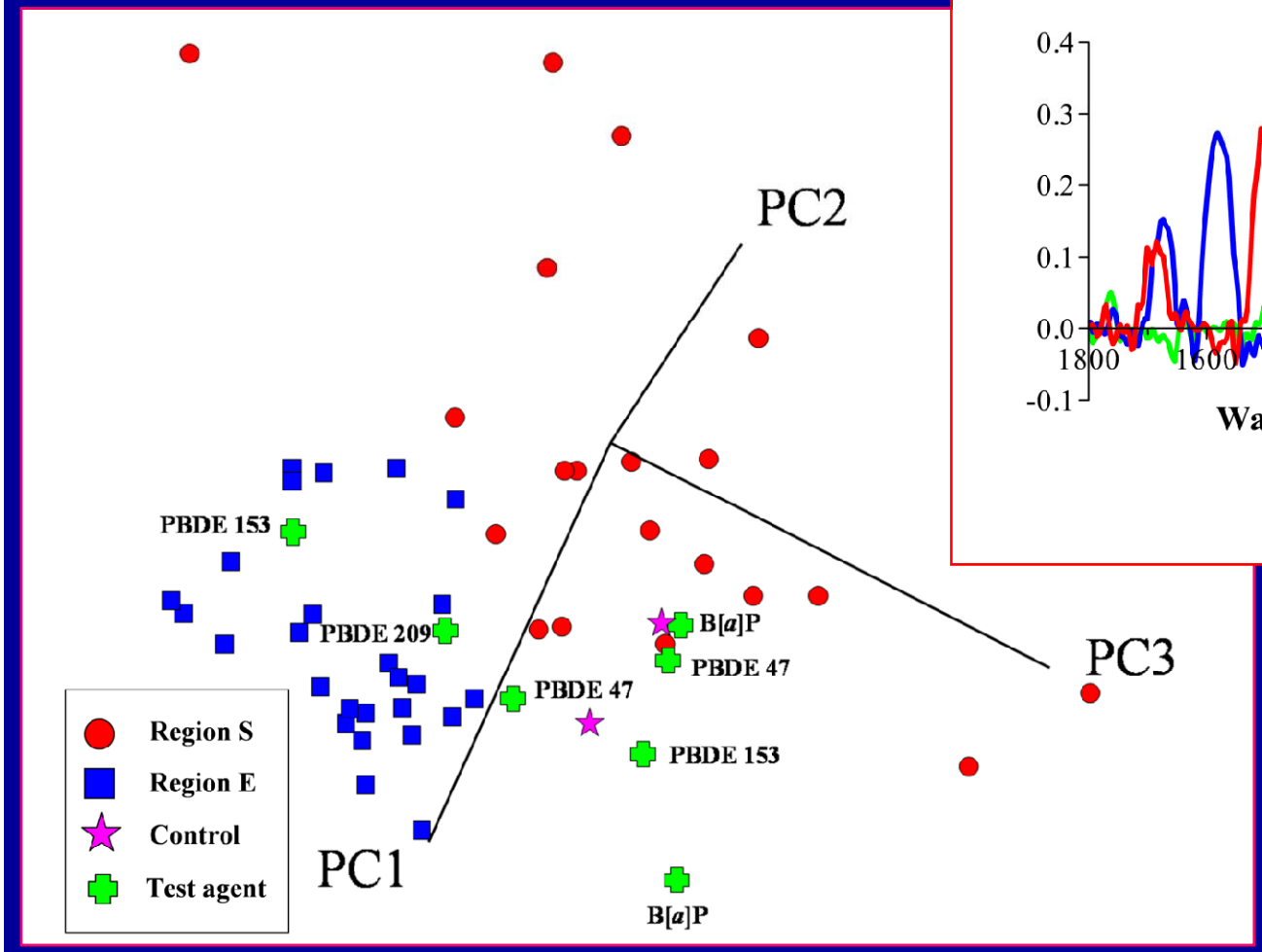


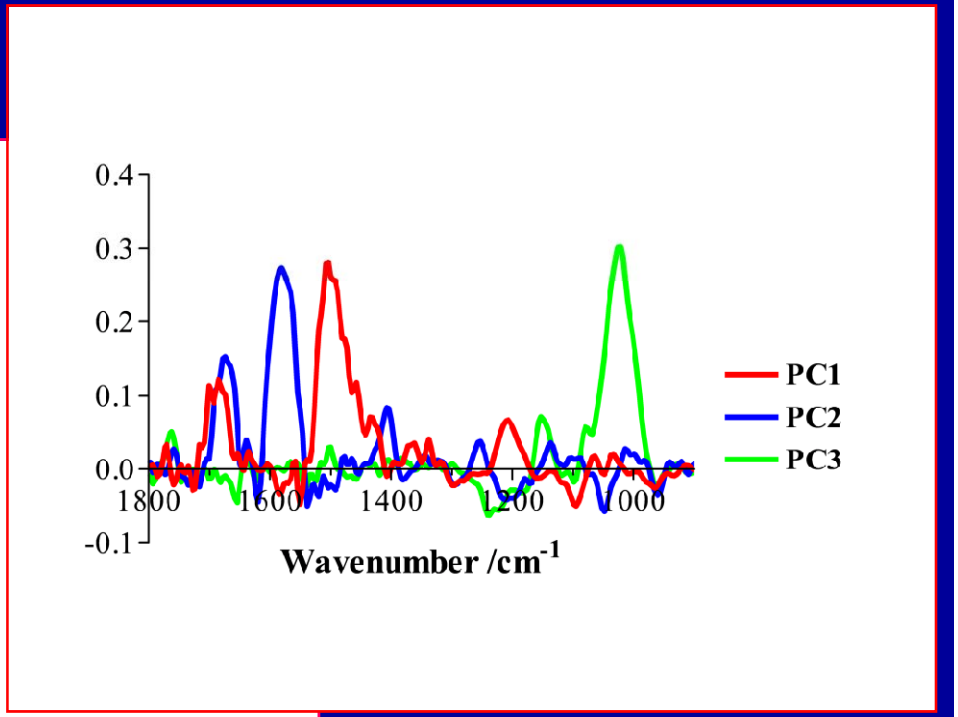

**Average IR spectra of MCF-7 cells following 24-h treatment with serum-lipid extracts from residents living in an e-waste dismantling region (Region E, n=26) *vs.* a region associated primarily with fishing industry (Region S, n=20)**

# In scores plot, data clusters according to exposure



# Additionally, co-segregates with positive controls



# Predatory Bird Monitoring Scheme (PBMS)

Predatory Bird Monitoring Scheme

- **Monitors exposure to contaminants of concern**

- **Polybrominated flame retardants**
- **Second generation rodenticides**
- **Polychlorinated biphenyls (PCBs)**
- **Mercury**
- **Organochlorine insecticides**

- **My study - information about effects**
- **First in birds**

# Species monitored by PBMS?





**Carcasses obtained from sparrowhawk, heron, barn owl, kestrel, red kite**





**Eggs collected from merlin, gannet, peregrine, golden eagle and sea eagle from nests (by licensed egg collectors)**

# Signature effects using IR spectroscopy

- **Cells treated with selected classes of chemicals**
- **Investigated dose-related effects (pM to μM)**
- **Polybrominated diphenyl ethers (PBDE congeners 47, 153, 183 and 209)**
- **Benzo[*a*]pyrene (B[*a*]P); 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PHIP)**
- **17β–Oestradiol ($E_2$); Lindane (γ-HCH)**

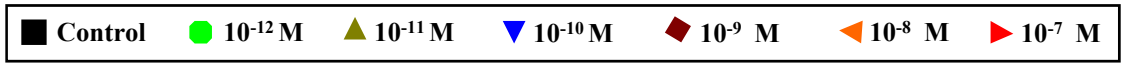
**Llabjani *et al.* (2009) ES&T; doi:10.1021/es8036127**

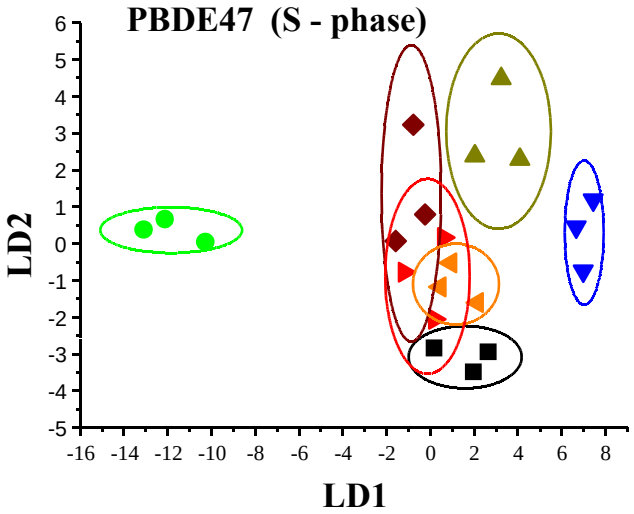
# My findings

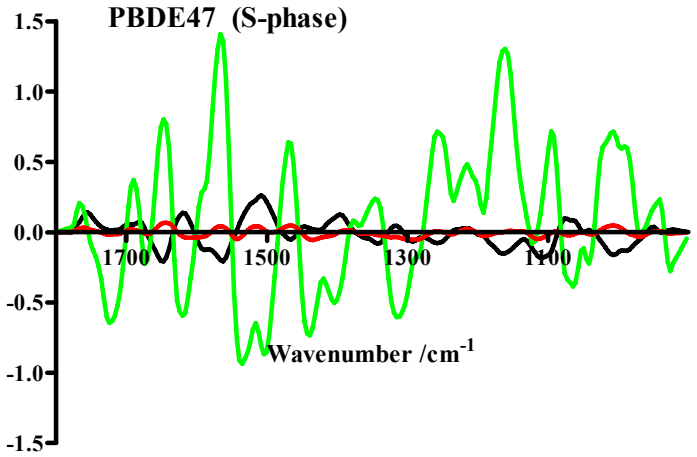
| Compound | Signature effects | Main identified biochemical markers |
|---|---|---|
| PBDEs congeners 47, 153, 183 and 209 | ✓ | C=O stretching vibrations of lipids (≈1750 $cm^{-1}$) Amide II alterations (≈1550 $cm^{-1}$) changes in lipid and protein secondary structure |
| Endocrine-active compounds (17β–Oestradiol, lindane) | ✓ | Amide I (≈1650 $cm^{-1}$) and Amide II alterations (≈1550 $cm^{-1}$) - changes in protein secondary structure |
| Genotoxins (PHIP, B[*a*]P) | ✓ | DNA/RNA region (≈1080, ≈ 1225) $cm^{-1}$) Changes in DNA/RNA |

**? Signature effects associated with exposure to different chemical classes**

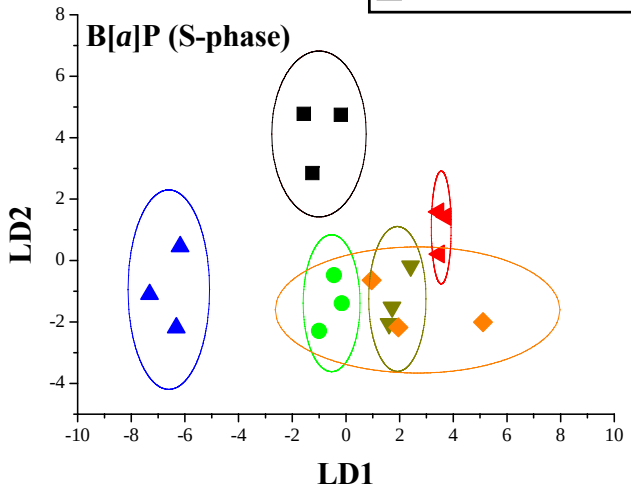
# PBDE47 *vs.* DNA-reactive B[*a*]P

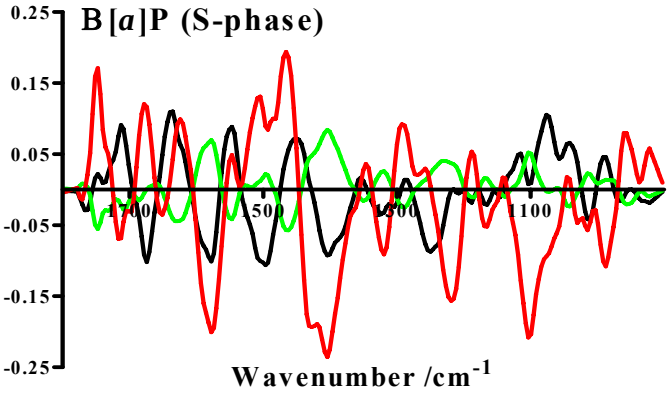






# Summary

- A powerful new technology platform
- Cluster biomarkers of effects according to exposure
- Extremely sensitive approach – identify pM to μM effects

# Future work

- Extracts of archived samples will be tested to generate a database of chemical class-specific effects
- Can effects signatures predict exposure patterns?
- Use archived material to validate the approach
- Develop a high-throughput fingerprinting methodology independent of expensive analytical approaches

# Recent publications

- Valon Llabjani, Kevin C. Jones, Gareth O. Thomas, Lee A. Walker, Richard F. Shore and Francis L. Martin (2009) Polybrominated Diphenyl Ether-Associated Alterations in Cell Biochemistry as Determined by Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy: a Comparison with DNA-Reactive and/or Endocrine-Disrupting Agents. Environmental Science & Technology ; doi: 10.1021/es8036127

# Acknowledgements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JOINT NATURE CONSERVATION COMMITTEE

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